

## Biomolecular Surfaces that Release Ligands under Electrochemical Control

Christian D. Hodneland and Milan Mrksich\*

Department of Chemistry, University of Chicago  
Chicago, Illinois 60637

Received February 3, 2000

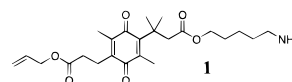
The design of any material that will contact a biological environment requires that the surface of the material be tailored to have desired interactions with molecules, proteins, or cells of the contacting biological fluid. Applications that require precise control over these interactions have benefited from the use of self-assembled monolayers (SAMs) of alkanethiolates on gold because these structurally ordered films offer unprecedented flexibility in modifying surfaces with ligands and other moieties. These characteristics were important in developing monolayers that are inert in biological fluids—in that they prevent protein adsorption and cell adhesion—which provided methods for patterning the positions and shapes of attached cells.<sup>1</sup> The attachment of ligands to these inert SAMs gives surfaces to which proteins and other receptors selectively bind. Monolayers presenting peptide ligands, for example, have been used to control the adhesion of cells,<sup>2</sup> and monolayers presenting oligonucleotides have been used for probing gene expression in cells.<sup>3</sup>

A new challenge in biointerfacial science is to design *dynamic* substrates that can alter, in real-time, the display of ligands and, hence, the interactions of proteins and cells with the substrate.<sup>4</sup> We previously demonstrated a dynamic SAM that could be switched from a state that is initially inert to a state that permits the Diels–Alder mediated immobilization of ligands, which in turn provides a strategy to activate the selective binding of proteins to a substrate.<sup>5</sup> Here, we describe a new class of dynamic electroactive monolayer that can selectively *release* immobilized ligands.

The monolayer shown in Figure 1 was designed to release the ligand biotin when a reductive potential is applied to the underlying gold. This dynamic property derives from the quinone propionic ester that tethers the biotin to the monolayer. Previously, quinone propionic esters and amides have been used as protecting groups for alcohols and amines, respectively, because mild chemical reduction of the quinone affords the hydroquinone, which rapidly lactonizes with liberation of an alcohol or amine.<sup>6</sup> The two methyl groups at the benzylic position together with the proximal methyl group on the ring—collectively referred to as the “tri-methyl lock”—serve to increase the rate of the lactonization reaction, and therefore, the release of ligand.<sup>7</sup> For our purposes it is essential that the monolayers remain inert to the non-specific adsorption of protein—both before and after release of the ligand. Accordingly, the monolayers used here present the electroactive tether at low density (approximately 1% of total alkanethiolate) surrounded by tri(ethylene glycol) groups because

the latter are highly effective at preventing non-specific adsorption of protein.<sup>8</sup>

We first established that the quinone propionic ester moiety could undergo *electrochemical* reduction and subsequent lactonization to give the desired products in high yield. We prepared a model quinone propionic ester (**1**) and electrolyzed a solution of this molecule (1 mM, 5 mg total in 1:1 THF:H<sub>2</sub>O containing 200 mM KCl) at  $-900$  mV (all potentials reported here are relative to a Ag/AgCl/KCl reference) until the current decayed to 1% of its initial value.<sup>9</sup> Isolation and characterization of the reaction products showed that **1** was converted to the corresponding lactone and alcohol in quantitative yield.<sup>10</sup>



We next describe release of biotin from the monolayer shown in Figure 1. We used surface plasmon resonance (SPR) spectroscopy to measure the biospecific association of streptavidin with the monolayer and to demonstrate the loss of binding after biotin was electrochemically released.<sup>11,12</sup> The following sequence was used to measure the association of proteins to the monolayers: buffer (PBS, pH 7.4) was flowed over the monolayer for 3 min to establish a baseline; a solution of protein in the same buffer was flowed over the surface for 8 min to observe binding; buffer was again flowed for 4 min to quantitate the amount of protein that remained bound. Figure 2A shows that streptavidin (60 nM) bound to a monolayer presenting the quinone propionic ester substituted with biotin.<sup>13</sup> The change in resonance angle ( $\Delta\theta$ ) after streptavidin was flowed over the substrate corresponds to a final protein density of 1.1 ng/mm<sup>2</sup>. The protein remained irreversibly bound because of the high affinity of the streptavidin–biotin complex. When the streptavidin was mixed with biotin (140  $\mu$ M) before introduction to the SAM, there was no binding of the protein to the surface, demonstrating that the interaction is biospecific (Figure 2B). The reduction and subsequent lactonization and release of biotin was triggered by application of a potential of  $-700$  mV for 3 min.<sup>14,15</sup> SPR showed that the amount of streptavidin that bound to the resulting SAM decreased by 95% (Figure 2C). Importantly, the electrochemical treatment did not damage the monolayer or compromise its resistance to non-specific adsorption of several proteins, including the “sticky”

(8) Mrksich, M.; Whitesides, G. M. *ACS Symp. Ser. Chem. Biol. Appl. Polyethylene Glycol* **1997**, 680, 361–373.

(9) Bulk electrolysis was performed under an argon atmosphere using a BAS CV-50W potentiostat in a standard cell with a vitreous carbon working electrode of large surface area, a coiled platinum wire counter electrode, and a Ag/AgCl/KCl reference electrode.

(10) The reaction mixture was purified using column chromatography to afford the lactone, which was characterized by <sup>1</sup>H and <sup>13</sup>C NMR and TLC.

(11) For examples of the use of SPR to measure biospecific association of proteins with SAMs, see: (a) Houseman, B. T.; Mrksich, M. *Angew. Chem., Int. Ed.* **1999**, 38, 782–785. (b) Mrksich, M.; Grunwell, J. R.; Whitesides, G. M. *J. Am. Chem. Soc.* **1995**, 117, 12009–12010. (c) Spinke, J.; Liley, M.; Guder, H. J.; Angermaier, L.; Knoll, W. *Langmuir* **1993**, 9, 1821–1825.

(12) Monolayers were prepared by immersing gold-coated glass slides in ethanolic solutions containing a mixture of a symmetric disulfide of an alkanethiol substituted with tri(ethylene glycol) and an unsymmetric disulfide of an alkanethiol substituted with the tri(ethylene glycol) and the biotin quinone propionic ester. The unsymmetric disulfide was synthesized in 29 steps from commercially available reagents. All intermediates gave satisfactory <sup>1</sup>H NMR and MS spectra.

(13) SPR measures the angle of light ( $\theta$ ) reflected from the backside of the gold substrate that is a minimum in intensity. Changes in this angle ( $\Delta\theta$ ) are linearly related to the index of refraction of the solution above the surface and therefore to the density of adsorbed protein ( $\Delta\theta$  of 0.10° = 1 ng/mm<sup>2</sup>). Experiments show a change in  $\theta$  immediately following protein injection due to differences in refractive index between the two solutions.

(14) Electrochemistry was performed in buffered water (PBS, pH 7.4) using the gold substrate as the working electrode, a platinum wire as the counter electrode, and a Ag/AgCl/KCl reference electrode—prior to mounting the substrate in a cartridge for analysis by SPR.

(1) (a) Mrksich, M.; Dike, L. E.; Tien, J. Y.; Ingber, D. E.; Whitesides, G. M. *Exp. Cell Res.* **1997**, 235, 305–313. (b) Chen, C. S.; Mrksich, M.; Huang, S.; Whitesides, G. M.; Ingber, D. E. *Science* **1997**, 276, 1345–1347.

(2) (a) Roberts, C.; Chen, C. S.; Mrksich, M.; Martichonok, V.; Ingber, D. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1998**, 120, 6548–6555. (b) Houseman, B. T.; Mrksich, M. *J. Org. Chem.* **1998**, 120, 6548–6555.

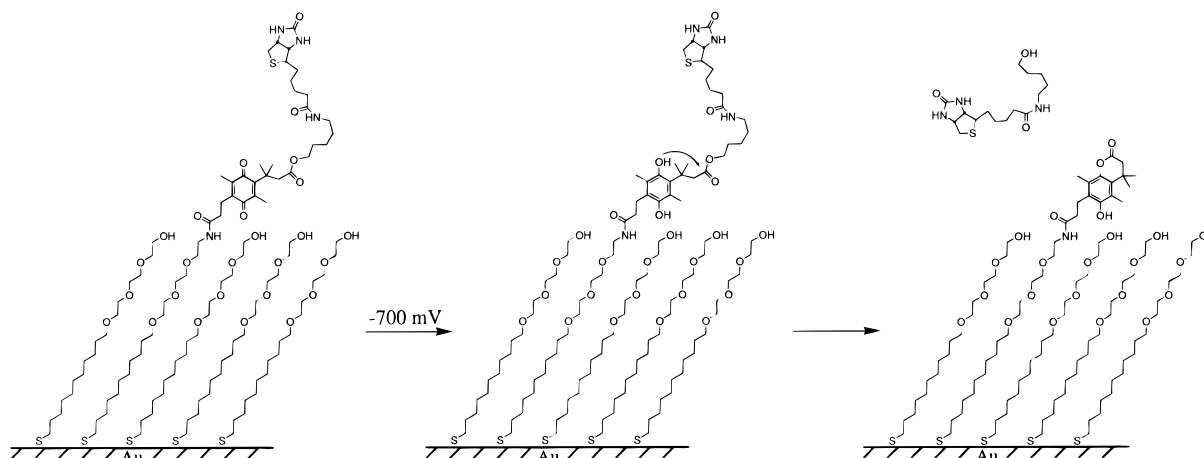
(3) Bamdad, C. *Biophys. J.* **1998**, 75, 1997–2003.

(4) Early examples of materials with dynamic function include: (a) Lau, A. N. K.; Miller, L. L. *J. Am. Chem. Soc.* **1983**, 105, 5271–5277. (b) Ding, Z.; Long, C. J.; Hayashi, Y.; Bulmus, E. V.; Joffman, A. S.; Stayton, P. S. *Bioconjugate Chem.* **1999**, 10, 395–400.

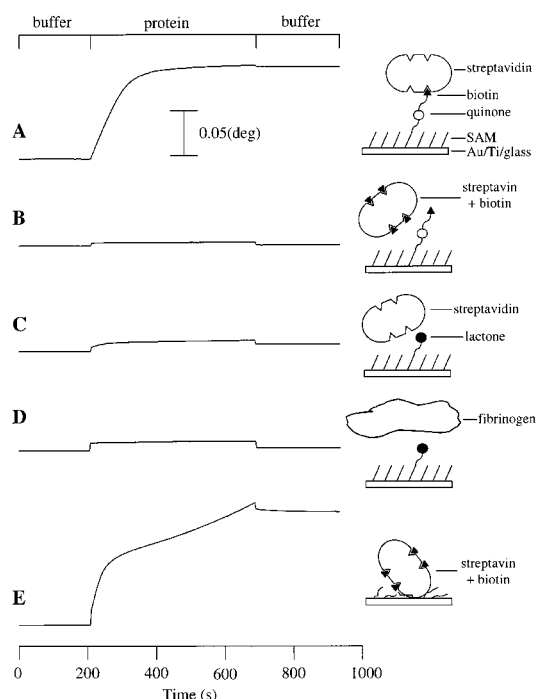
(5) Yousaf, M. N.; Mrksich, M. *J. Am. Chem. Soc.* **1999**, 121, 4286–4287.

(6) (a) Carpino, L. A.; Triolo, S. A.; Berglund, R. A. *J. Org. Chem.* **1989**, 54, 3303–3310. (b) Wang, B.; Liu, S.; Borchardt, R. T. *J. Org. Chem.* **1995**, 60, 539–543. (c) Zheng, A.; Shan, D.; Wang, B. *J. Org. Chem.* **1999**, 64, 156–161.

(7) Milstien, S.; Cohen, L. A. *J. Am. Chem. Soc.* **1972**, 94, 9158–9165.



**Figure 1.** Design of a self-assembled monolayer (SAM) that selectively releases the ligand biotin upon application of a reductive potential. The ligand is tethered to the alkanethiolate through a quinone propionic ester and is present at a density of 1%. On application of a potential of  $-700$  mV to the underlying gold film, the quinone is reduced to the hydroquinone, which then undergoes rapid lactonization with release of biotin. The tri(ethylene glycol) groups in the monolayer prevent the non-specific adsorption of protein.



**Figure 2.** SPR data showing the association of proteins with the SAMs (see text for an explanation of the data). The change in resonance angle ( $\Delta\theta$ ) is plotted on the vertical axis: the scale bar applies to all data, which are offset for clarity. (A) Streptavidin (60 nM) associated with a surface presenting biotin. (B) Streptavidin pre-incubated with biotin (140  $\mu$ M) showed no association with the surface, demonstrating that the interaction is biospecific. (C) Application of a potential of  $-700$  mV for 3 min to the monolayer, prior to the binding experiment, resulted in the release of ligand and a 95% reduction in streptavidin binding. (D) Fibrinogen (0.5 mg/mL) did not adsorb to the SAM following electrochemical treatment, demonstrating that the SAM remained inert to protein adsorption. (E) Application of a potential of  $-1100$  mV for 5 min caused association of streptavidin presaturated with biotin, showing the surface was damaged and was no longer resistant to non-specific protein adsorption.

protein fibrinogen (Figure 2D).<sup>16</sup> As a final control, we prepared a monolayer presenting biotin but that did not incorporate the

(15) Ellipsometric characterization of a monolayer that presented the biotin quinone propionic ester at a density of 25% (to increase signal contrast) showed that the thickness decreased by 5 Å after electrochemical treatment, consistent with release of biotin from the surface. Grazing-angle FTIR spectra were inconclusive, presumably because of the disordered structure of the oligo(ethylene glycol) groups and the biotin quinone propionic ester groups.

quinone propionic ester tether. For this monolayer, application of a potential of  $-700$  mV for 3 min had no effect on the amount of streptavidin that associated, and the monolayer also remained resistant to non-specific protein adsorption (data not shown). These data establish that ligands tethered to a monolayer with the quinone propionic ester can be selectively released without compromising the inertness of the monolayer or the activity of ligands that are not tethered to the monolayer through the quinone propionic ester.

The dynamic substrate described here embodies a general strategy for preparing biosurfaces that offer unprecedented, real-time control over the properties and functions of biosurfaces. Central to this strategy is the structural order inherent to SAMs, which allow the structures and environments of ligands to be tailored with molecular-scale control. Also important is the use of inert SAMs that prevents non-specific interactions at the interface. The incorporation of appropriate redox-active moieties that can be electrochemically switched by applying potentials to the gold provides a non-invasive and selective means to modify the structure and properties of the monolayer. This strategy to manipulate interfacial properties has the advantage that it is less invasive than alternate strategies that use heat, light, or reagents to alter properties. Electrically active films also offer advantages for efficient integration with microelectrical systems, including the use of microelectrode arrays and patterned monolayers to build surfaces that combine multiple functions.

We believe that this method will be most important for engineering tailored substrates for mechanistic studies in cell biology. The interactions of adherent cells, *in vivo*, with the insoluble extracellular matrix are mediated by the binding of cell-surface receptors to peptide and carbohydrate ligands of the matrix. Many cellular functions—including metastasis and migration—are regulated by changes in the composition of ligands present in the matrix. The surface chemistry approach described here makes possible the preparation of substrates that present multiple ligands, but which can be triggered to selectively release a single class of ligand, and will provide new opportunities in experimental cell biology.

**Acknowledgment.** We are grateful for support provided by the National Science Foundation (BES-9980850) and the Materials Research Science and Engineering Center (DMR-9808595).

JA000419P

(16) The use of more extreme potentials ( $-1100$  mV for 5 min) gave monolayers that were no longer inert to non-specific protein adsorption (Figure 2E), and lower potentials ( $-600$  mV for 3 min) gave incomplete cleavage of biotin.